**BG4 Indirect Immunofluorescence Protocol**

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1. Seed cell line of choice on sterile coverslips the night before harvest so that they will be 50% to 80% confluent. Use 24mm (circular coverslips) pre-coated with 0.1mg/mL poly-D-lysine (Sigma, cat. no. P7405) in the bottom of a 12-well plate.
2. Fix using 1mL of 4%paraformaldehyde in 1 x PBS for 10 min at RT.
3. Rinse carefully with 2mL PBS.
4. Permeabilise with 1 x PBS containing 0.1% Triton-X for 10 min at RT.
5. Rinse x2 with PBS .
6. *Optional: RNase A treat coverslips with 20 L 10mg/mL RNaseA + 800 L PBS: (50 L per coverslip). Incubate for 1 h at 37 degrees. This should reduce any cytoplasmic foci to background and leave the majority of nuclear foci untouched.*
7. *Optional: DNase treat cells using 60U Turbo DNase (ThermoFisher cat. no. AM2238) in 1 x Turbo DNase buffer (50 L per coverslip) and incubate at 37 degrees for 1 h. This should remove most nuclear foci and not touch cytoplasmic foci.*
8. Block coverslips for 1h with 0.5% goat serum in PBST (PBS + 0.1% Tween20) at 37 degrees.
9. Incubate coverslips with the BG4 antibody diluted (1:100 in 0.5% goat serum + PBST (50L per coverslip) and incubate at 37 degrees for 1 h. We suggest that, for optimal results, the antibody is titred; typically the stock of BG4 antibody that we make is ~6-8M.
10. Wash three times with PBST for 5 min each.
11. Incubate with 1:800 Rabbit anti-FLAG (cat. no. 2368, Cell Signaling Technology) in PBST + 0.5% goat serum for 1 h at 37 degrees (50L per coverslip).
12. Wash three times with PBST for 5 mins each.
13. Incubate with secondary antibody diluted in in PBST + 0.5% goat serum for 1 h at RT e.g. Rabbit Alexa Fluor 594 1:500- 1:1000 (ThermoFisher cat. no. A11037).
14. Wash three times with PBST for 5 min each (second wash containing DAPI).
15. Rinse 3 times with RO water.
16. Air dry.
17. Mount coverslips on Superfrost Plus slides (cat. no. 4951PLUS) with ThermoFisher ProLong Diamond anti-fade mounting medium (cat. no. P36970).

Notes:

1. We only recommend the single chain phage display version. The pSANG10-3F-BG4 expression plasmid is available from Addgene (Plasmid #55756)and the antibody is available from Merck in limited quantities (cat. no MABE917). We have not tested the BG4 format available from Absolute Antibody.
2. Methanol/acetic acid may be a better fixative for some cell types/species.
3. We find that the use of goat serum rather than milk allows detection of weaker signals.